Evidence of Cancer Promoting Roles for AMPK and Related Kinases

Tiziana Monteverde[1], Nathiya Muthalagu[2], Jennifer Port[1] and Daniel J. Murphy*[1,2],

[1] Institute of Cancer Sciences, University of Glasgow, U.K.

[2] CRUK Beatson Institute, Glasgow G61 1BD, U.K.

* To whom correspondence should be addressed at

daniel.murphy@glasgow.ac.uk

+44 141 330 8710

Abstract:

The discovery that the 5'AMP-activated protein kinase, AMPK, serves to link the tumour suppressors LKB1 and the Tuberous Scelorsis Complex (TSC), and functions to slow macromolecular synthesis through attenuation of the mechanistic Target of Rapamycin Complex 1 (mTORC1), revealed a role for AMPK in tumour suppression. On the other hand, the well-recognized role of AMPK in maintaining ATP homeostasis, through suppression of anabolism and promotion of catabolism, as well as the role of AMPK in neutralising reactive oxygen species (ROS), via maintenance of NADPH-dependent reductive capacity, point to tumour-protective roles in the context of metabolic stress, which is a key feature of many solid tumours. A growing number of studies thus suggest a duality of functions for AMPK that are either pro- or anti-cancer, depending upon context. Importantly, AMPK is comprised of 3 subunits and multiple isoforms exist for all three, allowing for different permutations to assemble and the potential for specific AMPK complexes to regulate distinct cellular processes. Moreover, certain subunits of the AMPK complex are frequently overexpressed in a spectrum of human cancer types, suggesting an outright oncogenic function for specific AMPK complexes. Adding complexity to this picture, the catalytic AMPK alpha subunits belong to a family of 14 kinases that can all be activated by LKB1 and studies are beginning to reveal a similar duality of roles in cancer for other members of the AMPK-related kinase family.

Cancer cells divert enormous resources into fuelling the growth required to sustain their unscheduled proliferation. Commonly arising oncogenic mutations resulting in RAS and PI3K pathway activation, p53 inactivation or MYC overexpression, directly impinge upon core cellular metabolism, at once driving proliferation and at the same time signalling to cells to redirect the breakdown products of nutrients into the synthesis of macromolecules required for cell growth [1, 2]. This diversion of nutrients comes at a cost however and cancer cells must continuously rebalance their rate of macromolecular synthesis and cell growth with the energetic cost of supporting that growth, measured in ATP. The fragility of this balancing act is underscored by the observation that cancer cells often exhibit exquisite sensitivity to nutrient deprivation, rapidly undergoing cell death where non-transformed counterparts respond by downregulating proliferative signalling and undergoing arrest [3-6]. In the context of a growing solid tumour, cancer cells are continuously exposed to a range of patho-physiological metabolic strains, including nutrient limitation, hypoxia and microenvironment acidification, owing to the inefficient and disorganised nature of the tumour vasculature. Indeed, poorly vascularised tumour regions typically show high levels of necrotic cell death [7]. Strategies to exploit the intrinsic metabolic vulnerabilities of tumour cells are thus now gaining in credibility and may have broad utility in the treatment of a spectrum of cancers [8-11].

AMPK Maintains ATP Homeostasis

The 5'AMP-activated protein kinase, AMPK, is a key regulator of the balance between cell growth and bioenergetic homeostasis. In general, AMPK promotes processes that generate or preserve cellular ATP, including glycolysis, oxidative phosphorylation, β-oxidation of fatty acids and autophagy, and inhibits processes that consume ATP, such as protein translation, ribosome assembly and lipid synthesis [12, 13]. As its name suggests, AMPK activity increases with rising [AMP], or more precisely, upon an increase in the cellular [AMP]:[ATP] ratio. AMPK is a trimeric complex comprised of a catalytic alpha subunit and a regulatory gamma subunit held together by a scaffolding beta subunit. The gamma subunit can bind up to 3 molecules of AMP, at least 2 of which can exchange for ATP which reduces activity, thereby allowing AMPK to directly detect changes in the [AMP]:[ATP] ratio [14]. This ability to simultaneously bind activating and inhibitory adenosine phosphate residues ensures a graded rather than binary response to the cellular metabolic state, enabling cells to continuously "fine-tune" their rate of macromolecular synthesis in line with energetic fluctuations. AMPK is thus activated indirectly by a wide variety of compounds that increase the cellular

[AMP]:[ATP] ratio, such as biguanides Metformin and Phenformin, mitochondrial toxins and modulators, 2-deoxyglucose, and indeed by nutrient deprivation [15]. The importance of AMPK's role in ATP homeostasis is underlined by the fact that many such compounds are profoundly toxic in cells that lack functional AMPK, yet are well tolerated by AMPK-expressing counterparts [16].

AMPK in Protection from Reactive Oxygen Species

Reactive oxygen species (ROS) are produced primarily as a natural by-product of mitochondrial respiratory chain activity [17]. While moderate levels of ROS, in particular H_2O_2, participate in signal transduction, high levels of ROS can result in macromolecular damage and cytotoxicity. ROS levels are elevated by impaired mitochondrial function, driven by mitochondrial mutations, oncogenic signalling and, importantly, by hypoxia [18]. Notably, AMPK is activated by hypoxia in a ROS-dependent manner [19] and is implicated in hypoxia-driven angiogenesis [20]. Treatment of cells with exogenous H_2O_2 likewise activates AMPK [21]. AMPK was shown to play a key role in cellular antioxidant defence by preserving NADPH levels, via inhibition of ACC1/2-mediated fatty acid synthesis and activation of fatty acid oxidation [22]. NADPH is a major antioxidant required for maintaining the reductive capacity of glutathione and lowering ROS levels, thereby protecting cells from oxidative stress-induced death [23]. Consistent with a tumour-promoting role for AMPK-mediated ROS defence, the tumour suppressor Folliculin was recently identified as a negative regulator of AMPK, and loss of Folliculin was shown to protect cells from death induced by H_2O_2, amongst other stresses, through an evolutionarily conserved mechanism involving AMPK-dependent activation of autophagy [24, 25].

The Paradox of Tumour Suppression by AMPK

The picture that emerges is that AMPK plays a central role in the adaptive responses to cellular metabolic stress. This ability to respond dynamically to a spectrum of metabolic insults is of obvious benefit to tumour cells in a hostile microenvironment, where nutrients, growth factors and oxygen are limiting, while metabolic waste accumulates, as tumours outgrow their vascular supply. Thus AMPK may be critical for maintaining cancer cell viability in established tumours, making it an attractive target for pharmacological inhibition. Somewhat paradoxically however, one mechanism by which AMPK can preserve ATP is through the inhibition of mTORC1-driven protein translation [26, 27], and this very activity has fuelled the notion that AMPK can function as a tumour suppressor, given that mTORC1

activity is increased in cancer via activation of upstream oncogenic signalling through PI3K and AKT and/or loss of upstream tumour suppressors PTEN, TSC and LKB1. The discovery that LKB1 directly activates AMPK, thereby linking LKB1 to suppression of mTORC1, and that AMPK in turn activates the Tuberous Sclerosis Complex (TSC), another negative regulator of mTORC1, seemed to place AMPK squarely in a tumour suppressive role [28-31]. More, recently, the discovery that MAGE A3/6 targets AMPKα1 for degradation appears to buttress this interpretation: expression of MAGE A3/6 proteins is normally restricted to the testes but is reportedly widespread in human cancer [32]. Expression of MAGE A3/6 is sufficient to increase focus formation and anchorage-independent growth of immortalised cell lines. Expression of MAGE A3/6 moreover increases mTORC1 signalling and suppresses autophagy in a manner that requires AMPKα1 degradation, whereas depletion of MAGE A3/6 has the opposite effects. These observations collectively indicate that reducing AMPK activity can have tumour-promoting consequences. However, reducing AMPK activity is not the same as completely suppressing it, and a number of observations confound the simple designation of AMPK as a *bona-fide* tumour suppressor.

Firstly, although the predominant kinase upstream of AMPK in many cells, LKB1 is not the only kinase capable of activating AMPK and we, amongst several other groups [22, 33-35], observe robust activation of AMPK in LKB1-deficient tumour cells, such as A549 and HeLa, upon treatment with both direct (A769662, Salicylate) and indirect (2DG, Phenformin, Ca^{++} Ionophore) AMPK stimuli (Figure 1). Indeed, the TGFβ-activated kinase TAK1 and the Ca^{++}/Calmodulin-dependent kinase CaMKKβ have both been shown to directly phosphorylate the AMPKα subunit on the same T172 residue that is targeted by LKB1 [33, 36]. Activation of AMPK by CaMKKβ may be of particular relevance in the context of tumour hypoxia, as hypoxia-induced ROS has been linked to Ca^{++} release from the endoplasmic reticulum, leading to activation of CaMKKβ and AMPK [37]. Moreover the Androgen Receptor/CaMKKβ/AMPK axis has been proposed to play a prominent role in the etiology of prostate cancer [38]. Thus, loss of LKB1 does not necessarily equate with loss of AMPK activity.

Secondly, there is little evidence of AMPK deletion or inactivating mutation in human cancer. In mammals there are 2 genes each encoding alpha (*PRKAA1, PRKAA2*) and beta (*PRKAB1, PRKAB2*) subunits and three encoding gamma subunits (*PRKAG1, PRKAG2, PRKAG3*), and this genetic redundancy is often cited as a plausible explanation for the retention of wild type alleles [39]. On the contrary however, there is now clear evidence that specific AMPK subunits, notably *PRKAA1* (AMPKα1) and *PRKAB2* (AMPKβ2), are frequently amplified across a broad spectrum of human cancers (Figure 2)[40, 41]. This selective amplification is also

observed in established human tumour cell lines and correlates closely with elevated mRNA expression (Figure 3A)[42]. Amplification of *PRKAA1* and *PRKAB2* coincides significantly with activation and/or amplification of dominant oncogenes such as *KRAS*, *BRAF* and *AKT*, while *PRKAB2* amplification in particular coincides significantly with *MYC* amplification across several cancer types including Melanoma, Breast and Bladder cancers (Figure 3B & C and data available via cBioPortal). Although these data do not provide definitive evidence of an outright oncogenic role for these subunits, they clearly necessitate a rethink of the possible roles of AMPK in human cancer.

A third and related point is that "AMPK" refers not to one complex but potentially to many complexes: Not accounting for splice variants, the 7 genetically encoded AMPK subunits in principle allow for assembly of up to 12 distinct AMPK complexes. It is tempting to speculate that different AMPK complexes might selectively regulate specific cellular processes via distinct downstream effectors, or indeed respond differentially to specific upstream stimuli. There is already some evidence to suggest that this is the case: Deletion of *STK11*, (encoding LKB1) in cardiac myocytes suppresses activation of AMPKα2 and downstream phosphorylation of Acetyl-CoA Carboxylase 2 (ACC2) in response to ischemia, however, activation of AMPKα1 is unaffected – by inference AMPKα1 plays a minor role in the regulation of ACC2, at least in this context [43]. Additionally, FRET biosensors of AMPK activity directed to specific subcellular compartments reveal that plasma membrane-, lysosome- and golgi-associated AMPK complexes preferentially contain α1 over α2, suggesting a physical segregation of function for such complexes [44]. It is thus possible that certain AMPK complexes might promote tumour suppression while others favour tumour survival.

Finally, with the striking exception of Pulmonary Adenocarcinoma, where *STK11* is mutated in up to 20% of cases, genetic loss of LKB1 is relatively infrequent in sporadic human cancer (Figure 2). Although promoter methylation at the *STK11* locus has been reported in sporadic colorectal cancer, it appears to be a relatively rare event [45]. Thus, it would seem that the vast majority of human cancers retain the capacity to call upon a functional LKB1/AMPK pathway for protection in the face of metabolic stress. Consistent with this perspective, although deletion of *Stk11* profoundly accelerates KRas-driven tumours in a mouse model of lung cancer, such tumours are exquisitely sensitive to the mitochondrial inhibitor Phenformin [46]. Similarly, deletion of *Stk11* in an ErbB2-driven mouse model of breast cancer, and deletion of *Prkaa1* in Eμ-MYC-driven lymphoma, both accelerate tumourigenesis but in both instances render the tumour cells profoundly sensitive to

metabolic stress [47, 48]. Thus, even under circumstances where loss of the LKB1/AMPK pathway promotes tumour development, loss of this pathway simultaneously elicits a metabolic vulnerability that can potentially be exploited for therapy [49, 50].

Tumour promoting roles of AMPK-related Kinases (ARKs)

The alpha subunits of AMPK belong to an extended family along with another 12 related kinases: BRSK1, BRSK2, MARK1 (PAR-1c), MARK2 (PAR-1b), MARK3 (PAR-1a), MARK4 (PAR-1d), MELK, NUAK1 (ARK5), NUAK2 (SNARK), SIK1, SIK2 (QIK) and SIK3 (QSK). All of the ARKs bar MELK can be phosphorylated by LKB1 [29], although for some ARKs additional kinases are implicated as upstream regulators [51-54]. Several of the family members are broadly conserved across evolution, even as far as the plant kingdom [55], indicative of the ancient origin of these proteins and their crucial importance for most life forms on Earth. While our understanding of the ARKs lags some distance behind that of AMPK itself, to generalise, the physiological roles of these kinases fall into three categories: regulation of cell polarity; regulation of cell migration and regulation of metabolism at both cellular and organismal levels.

NUAK1

NUAK1 was initially isolated as the 5th AMPK-related mammalian kinase and hence is also termed ARK5. Early reports linked NUAK1 to AKT signalling and specifically to IGF-induced cell migration and invasion [56, 57]. NUAK1 was mechanistically linked to cell detachment via direct phosphorylation of the myosin phosphatase complex subunit MYPT1, supporting a role for NUAK1 in facilitating cell motility [58]. Reduced expression of miRNAs targeting NUAK1 is associated with invasion in Melanoma and metastasis in Head and Neck Squamous Cell Carcinoma [59, 60]. Additionally, NUAK1 has been identified as a risk factor in Ovarian cancer [61] and is mutated in a small percentage of Oesophageal cancers [62].

We recently identified NUAK1 in a synthetic lethal RNAi screen for kinases that are selectively required to support tumour cell viability when MYC is overexpressed [63], a result that was independently reproduced by the Goga lab [64]. The synthetic lethal interaction was observed using multiple distinct small interfering and short hairpin RNA sequences and could be rescued by genetic complementation using a non-targeted NUAK1 cDNA. Confirming these results, a recently described highly-selective small molecule inhibitor of NUAK1, HTH-01-015 [65], selectively kills MEFs when MYC is acutely deregulated (Figure 4). Acute MYC deregulation in cells depleted of NUAK1 results in ATP collapse, revealing an unexpected role

for NUAK1 in ATP homeostasis, at least in the context of MYC deregulation. The energetic stress induced upon suppression of NUAK1 isn't limited to the *in vitro* setting: We showed that depletion of NUAK1 suppressed tumour formation and extended survival in an orthotopic mouse model of MYC-driven Hepatocellular Carcinoma, suggesting that NUAK1 is a potential target for cancer therapy [63].

Functionally, we linked NUAK1 to maintenance of mitochondrial fitness, or rather to mitochondrial plasticity: MYC activation increases expression of specific respiratory chain components, thereby enhancing respiratory capacity [66], and this adaptive effect was abrogated upon depletion of NUAK1. Moreover, we also exposed an unexpected role for NUAK1 in MYC-dependent activation of AMPK and found that depletion of NUAK1 resulted in enhanced activity of mTORC1. Importantly, AMPKα1 was also identified in our original synthetic lethal with MYC screen (while AMPKβ1 was identified in an independent synthetic lethal with MYC screen [67]) and the synthetic lethal effect of depleting either AMPKα1 or NUAK1 was rescued by slowing ATP consumption, via inhibition of mTORC1 with Rapamycin. NUAK1 depleted tumour cells thus have reduced ATP-generating capacity and elicit an impaired AMPK response to MYC. Our data therefore also implicate AMPK as having a tumour-protective role in MYC-overexpressing cells, which at first glance appears to conflict with evidence that loss of AMPK accelerates Eμ-MYC-driven lymphomagenesis [48]. A plausible explanation is that blood-borne cancers are unlikely to be subject to the same metabolic stress encountered by solid tumours. Consistent with this possibility, the Jones group showed that AMPK-deficient Eμ-MYC lymphoma cells are extremely sensitive to metabolic stress whereas AMPK-replete counterparts are much more resistant [48]. Thus, a mutation that offers a selective advantage at the time of tumour initiation may become a liability later in malignancy, particularly in the context of solid tumours.

NUAK2

Closely related to NUAK1, NUAK2 (aka SNARK) is frequently amplified across a spectrum of human cancers (Figure 5), forming part of the 1q32 amplicon common in Melanoma, Glioblastoma and Mammary cancers [68-70] and a specific role for NUAK2 in Acral Melanoma has been proposed [69]. Co-amplification of the potent p53 suppressor MDMX (encoded by *MDM4*) along with the RAS-pathway effector ELK4 [71] complicates interpretation of the significance of NUAK2 amplification. However, numerous expression analyses accessible through Oncomine do indicate frequent overexpression of NUAK2 mRNA in human cancers, suggesting a potential role in disease maintenance. Similar to AMPK, NUAK2 activity was

shown to increase upon nutrient deprivation or H_2O_2 treatment, suggesting a role for NUAK2 in protection from nutrient and oxidative stress [72]. On the other hand, mice constitutively haplo-insufficient for NUAK2 are sensitized to azoxymethane-induced colonic tumour formation, although it is unclear if this reflects an enterocyte-autonomous phenotype rather than a consequence of whole-body haploinsufficiency, and loss of NUAK2 heterozygosity in the tumours was not reported [73].

Microtubule Affinity Regulating Kinases (MARKs/PAR-1 proteins)

The MARK sub-family of kinases are implicated in cell motility and the physiological regulation of energy metabolism [52, 74]. Constitutive deletion of *MARK2*, *3* or *4* all result in hypermetabolic phenotypes of varying severity, increased Insulin sensitivity, and resistance to high-fat diet-induced obesity, suggesting that these proteins contribute systemically to diabetes [75-77], which is a well-recognised risk factor in many cancers [78]. *MARK1* is amplified in roughly 12% of Breast and Liver cancers and is co-amplified with NUAK2 across multiple cancers, likely reflecting a broader amplification of the Q arm of chromosome 1 (Figure 5). MARK1 and 4 were recently shown to co-ordinately mediate LKB1's ability to suppress epithelial to mesenchymal transition (EMT) via DIXDC-dependent inhibition of SNAIL expression [79]. As such, these kinases may play an important role in suppression of metastasis of certain cancers, however, their function in primary tumours was not addressed in this study.

MARK2 was recently found to be overexpressed in 23 of 77 primary NSCLC tumour samples relative to paired non-malignant tissue, and overexpression correlated with copy number gains and/or locus hypomethylation [80]. Analysis of the TCGA cohort [81] revealed overexpression of MARK2 in over 50% of NSCLC, irrespective of histological subtype, and overexpression correlated more with hypomethylation than with copy number gains, especially amongst the Squamous subtype. In NSCLC cell lines with high levels of MARK2 protein expression, depletion of MARK2 by RNAi suppressed proliferation and was associated with decreased WNT, HIF1α and MYC pathway activity, while high expression of MARK2 correlated with resistance to Cisplatin, as had been previously reported [82]. Intriguingly, the *Xenopus* homologues of MARK2 and MARK3 are important for both canonical and non-canonical WNT signalling [83] and the WNT pathway is widely activated in human cancer [84, 85].

Regulation of the Hippo Pathway

Several ARKs have recently been implicated as important regulators of the Hippo pathway, which controls organ size and is deregulated in many cancers (for excellent reviews see refs. [86, 87]). The Hippo pathway comprises a transcription module, made up of YAP1 and TAZ, which are negatively regulated by a kinase module, including the effector kinases LATS1 & 2 and their upstream activators MST1 & 2. NUAK1 and 2 were shown to be able to directly phosphorylate LATS1 leading to LATS1 degradation [88], which is predicted to result in increased YAP1/TAZ activity. On the other hand, MARK1 and 4 were shown to promote LATS1 activation by driving membrane re-localization of another Hippo factor, SCRIB, required for MST1/2-dependent phosphorylation of LATS1/2 and consequent inactivation of YAP1 [89]. Adding complexity to this picture, AMPK was recently shown by three independent groups to suppress YAP1 activity in response to energetic stress [90-92]. An earlier study showed LKB1-dependent regulation of YAP1, independent of either AMPK or LATS, suggesting that additional ARKs participate in regulation of this pathway (Nguyen). Clearly, the LKB1 pathway intersects the Hippo pathway at multiple levels (see Figure 6) and it will be fascinating to determine how this regulation is coordinated and indeed if it is bi-directional. As is the case for LKB1 signalling, the Hippo pathway appears to have both tumour promoting and tumour suppressive functions [87] and the role of both pathways in human cancer is likely to be highly context dependent.

Concluding Remarks

The duality of roles for AMPK in the adaptive response to metabolic stress, versus the attenuation of biosynthetic processes and cell growth, point to a complex and dynamic relationship with Cancer that defies restrictive designation as either "Oncogene" or "Tumour-Suppressor". Moreover, the term "AMPK" is itself deceptive in that it captures an as yet undetermined number of potential trimeric complexes. Hints are emerging that multiple distinct AMPK complexes co-exist in cells and a number of intriguing questions are on the cusp of investigation: How do different AMPK complexes respond to different signalling and metabolic cues? Do they regulate distinct biological processes via select downstream targets? Is their activity coordinated and does cross-talk between complexes occur? Is there similar coordination with and amongst the 12 AMPKα-related ARKs? A growing list of small molecules that directly bind to AMPK suggests that these complexes can be targeted pharmacologically, although the bias to-date has been for compounds with AMPK-activating potential [93]. Selective small molecule inhibitors of AMPK would be useful, at the very least as tool compounds, and in the right context may have therapeutic potential in light of AMPK's

tumour protective roles. There is moreover a clear need to develop reagents that can distinguish between complexes of different subunit composition, while conditional allelic mice promise to shed much light on the roles of individual AMPK subunits and ARKs in normal physiology and indeed in the context of Cancer. Disentangling the specific roles of this family of kinases is likely to yield many more surprises and intriguing insights relevant to Cancer, Metabolism, Physiology and beyond in the years to come.

Acknowledgements

The authors wish to apologise for the many excellent studies and reviews that we failed to cite for reasons of limited space. We are grateful to Jennifer Morton and Oliver Maddocks for careful reading of this manuscript. T.M is supported by a PhD studentship from the British Lung Foundation (BLF); N.M. is funded through Worldwide Cancer grant no. 1198; J.P. is supported by E.C Marie Curie Actions grant SERPLUC to D.J.M..

Conflict of Interest Statement

The authors declare no conflict of interest in the publication of this work.

Figure Legends

Figure 1

LKB1-independent activation of AMPK. Equal numbers of U2OS (LKB1 w/t), A549 (LKB1 deficient) and HeLa (LKB1 deficient) cells were treated with Salicylate (10mM), Phenformin (3mM), Calcium Ionophore (3μM) or DMSO vehicle control (vc) for 1hr, then lysed in RIPA buffer, fractionated by SDS-PAGE and blotted for the canonical AMPK target, phosphor-ACC1-Ser79. Note that A769662 (100μM) was used in lieu of Salicylate in HeLa cells. Consistent with published reports, acute activation of AMPK is unimpeded by the absence of LKB1, except in the instance of Phenformin treatment of HeLa cells.

Figure 2

Amplification of AMPK subunits in human cancer. Graphs accessible through cBioPortal (http://www.cbioportal.org) show the cumulative frequency of genetic alterations at the *STK11* (top), *PRKAA1* (middle) and *PRKAB2* loci, across a spectrum of human cancers. Green bars reflect the frequency of mutation (inclusive of activating and inactivating); red bars, the frequency of gene amplification; blue bars show the frequency of gene deletion and grey bars reflect multiple alterations at the same locus. Data are sourced from the TCGA, except where noted, and published TCGA cohorts are indicated with an asterisk. Other cohorts are 1) SU2C; 2) Broad; 3) Yale; 4) MSKCC; 5) AMC; 6) Mich; 7) BCCRC; 8) Genetech; 9) ICGC; 10) UHK; 11) JHU; 12) Pfizer; 13) BGI; 14) Cornell; 15) DFCRC; 16) TSP; 17) Sanger.

Figure 3

Specific Overexpression of *PRKAA1* and *PRKAB2* in established human cancer cell lines. A) The Cancer Cell Line Encyclopedia (http://www.broadinstitute.org/ccle/home) dataset reveals selective amplification of *PRKAA1* (α1) and *PRKAB2* (β2) that correlates with mRNA overexpression across a broad spectrum of cancer derived cell lines. *PRKAA2* (α2) and *PRKAB1* (β2) by comparison show little evidence of consistent copy number alterations. Gene copy numbers are graphed along the X-axis while mRNA expression levels are graphed along the Y-axis. B) Analysis of the CCLE dataset through cBioPortal shows the mutation spectra of individual cell lines, arrayed from left to right, analysed for the indicated genes, revealing co-incident amplification/mutation of *PRKAA1* and *PRKAB2,* both with each other and with selected dominant oncogenes, including *KRAS* and *MYC*. Note that the graphic is truncated from the right for visualization purposes. C) Statistical analysis of data from the graphic in (B) shows significant co-occurrence of *PRKAA1* and *PRKAB2* genetic alterations with those in

dominant oncogenes including KRAS and MYC. Similar results can be retrieved through analysis of primary tumour datasets for Breast, Ovarian and Pancreatic cancers amongst others, through cBioPortal.

Figure 4

Synthetic Lethality of MYC deregulation with pharmacological inhibition of NUAK1. Primary mouse embryo fibroblasts expressing MYC fused to the Estrogen Receptor ligand-binding domain ($MycER^{T2}$), rendering MYC activity dependent upon the synthetic ligand 4-hydroxy-Tamoxifen (4-OHT), were treated with 0, 25nM or 100nM 4-OHT and/or the selective NUAK1 inhibitor HTH-01-015 (10μM), and the surviving cultures were stained with crystal violet after 48hrs. V.C. = vehicle control.

Figure 5

NUAK2 is frequently amplified in human cancer. Mutation spectra of *NUAK2* (SNARK) and *MARK1* (PAR-1C) across human cancer types, as per Figure 2. Data are adapted from cBioPortal, sourced from the TCGA, except where noted, and published TCGA cohorts are indicated with an asterisk. Other cohorts are 1) SU2C; 2) Broad; 3) Yale; 4) MSKCC; 5) AMC; 6) Mich; 7) BCCRC; 8) Genetech; 9) ICGC; 10) UHK; 11) JHU; 12) Pfizer; 13) BGI; 14) Cornell; 15) DFCRC; 16) TSP; 17) Sanger.

Figure 6

Regulation of Hippo Signalling by AMPK & ARKs. Phosphorylation of the transcriptional co-activators YAP1 and TAZ by LATS1 or LATS2 leads to cytosolic sequestration, thereby suppressing TEAD-driven transcription. LATS1/2 are negatively regulated by NUAK1/2 but activated directly by AMPK and indirectly by MARK1/4 kinases, downstream of LKB1. AMPK has additionally been shown to directly phosphorylate YAP1, preventing its interaction with TEAD transcription factors.

References

1. Dang, C. V. (2013) MYC, metabolism, cell growth, and tumorigenesis, *Cold Spring Harbor perspectives in medicine.* **3**.
2. Boroughs, L. K. & DeBerardinis, R. J. (2015) Metabolic pathways promoting cancer cell survival and growth, *Nature cell biology.* **17**, 351-9.
3. Wise, D. R. & Thompson, C. B. (2010) Glutamine addiction: a new therapeutic target in cancer, *Trends in biochemical sciences.* **35**, 427-33.
4. Altman, B. J. & Dang, C. V. (2012) Normal and cancer cell metabolism: lymphocytes and lymphoma, *The FEBS journal.* **279**, 2598-609.
5. Labuschagne, C. F., van den Broek, N. J., Mackay, G. M., Vousden, K. H. & Maddocks, O. D. (2014) Serine, but not glycine, supports one-carbon metabolism and proliferation of cancer cells, *Cell reports.* **7**, 1248-58.
6. Maddocks, O. D., Berkers, C. R., Mason, S. M., Zheng, L., Blyth, K., Gottlieb, E. & Vousden, K. H. (2013) Serine starvation induces stress and p53-dependent metabolic remodelling in cancer cells, *Nature.* **493**, 542-6.
7. Egeblad, M., Nakasone, E. S. & Werb, Z. (2010) Tumors as organs: complex tissues that interface with the entire organism, *Developmental cell.* **18**, 884-901.
8. Schulze, A. & Harris, A. L. (2012) How cancer metabolism is tuned for proliferation and vulnerable to disruption, *Nature.* **491**, 364-73.
9. Doherty, J. R. & Cleveland, J. L. (2013) Targeting lactate metabolism for cancer therapeutics, *The Journal of clinical investigation.* **123**, 3685-92.
10. Gurpinar, E. & Vousden, K. H. (2015) Hitting cancers' weak spots: vulnerabilities imposed by p53 mutation, *Trends in cell biology.* **25**, 486-95.
11. Galluzzi, L., Kepp, O., Vander Heiden, M. G. & Kroemer, G. (2013) Metabolic targets for cancer therapy, *Nature reviews Drug discovery.* **12**, 829-46.
12. Hardie, D. G., Ross, F. A. & Hawley, S. A. (2012) AMPK: a nutrient and energy sensor that maintains energy homeostasis, *Nature reviews Molecular cell biology.* **13**, 251-62.
13. O'Neill, H. M., Holloway, G. P. & Steinberg, G. R. (2013) AMPK regulation of fatty acid metabolism and mitochondrial biogenesis: implications for obesity, *Molecular and cellular endocrinology.* **366**, 135-51.
14. Gowans, G. J. & Hardie, D. G. (2014) AMPK: a cellular energy sensor primarily regulated by AMP, *Biochemical Society transactions.* **42**, 71-5.
15. Hardie, D. G., Ross, F. A. & Hawley, S. A. (2012) AMP-activated protein kinase: a target for drugs both ancient and modern, *Chemistry & biology.* **19**, 1222-36.
16. Vincent, E. E., Coelho, P. P., Blagih, J., Griss, T., Viollet, B. & Jones, R. G. (2015) Differential effects of AMPK agonists on cell growth and metabolism, *Oncogene.* **34**, 3627-39.
17. Sullivan, L. B. & Chandel, N. S. (2014) Mitochondrial reactive oxygen species and cancer, *Cancer & metabolism.* **2**, 17.
18. Sabharwal, S. S. & Schumacker, P. T. (2014) Mitochondrial ROS in cancer: initiators, amplifiers or an Achilles' heel?, *Nature reviews Cancer.* **14**, 709-21.
19. Emerling, B. M., Weinberg, F., Snyder, C., Burgess, Z., Mutlu, G. M., Viollet, B., Budinger, G. R. & Chandel, N. S. (2009) Hypoxic activation of AMPK is dependent on mitochondrial ROS but independent of an increase in AMP/ATP ratio, *Free radical biology & medicine.* **46**, 1386-91.
20. Nagata, D., Mogi, M. & Walsh, K. (2003) AMP-activated protein kinase (AMPK) signaling in endothelial cells is essential for angiogenesis in response to hypoxic stress, *The Journal of biological chemistry.* **278**, 31000-6.
21. Choi, S. L., Kim, S. J., Lee, K. T., Kim, J., Mu, J., Birnbaum, M. J., Soo Kim, S. & Ha, J. (2001) The regulation of AMP-activated protein kinase by H(2)O(2), *Biochemical and biophysical research communications.* **287**, 92-7.
22. Jeon, S. M., Chandel, N. S. & Hay, N. (2012) AMPK regulates NADPH homeostasis to promote tumour cell survival during energy stress, *Nature.* **485**, 661-5.

23. Jeon, S. M. & Hay, N. (2015) The double-edged sword of AMPK signaling in cancer and its therapeutic implications, *Archives of pharmacal research.* **38**, 346-57.
24. Yan, M., Gingras, M. C., Dunlop, E. A., Nouet, Y., Dupuy, F., Jalali, Z., Possik, E., Coull, B. J., Kharitidi, D., Dydensborg, A. B., Faubert, B., Kamps, M., Sabourin, S., Preston, R. S., Davies, D. M., Roughead, T., Chotard, L., van Steensel, M. A., Jones, R., Tee, A. R. & Pause, A. (2014) The tumor suppressor folliculin regulates AMPK-dependent metabolic transformation, *The Journal of clinical investigation.* **124**, 2640-50.
25. Possik, E., Jalali, Z., Nouet, Y., Yan, M., Gingras, M. C., Schmeisser, K., Panaite, L., Dupuy, F., Kharitidi, D., Chotard, L., Jones, R. G., Hall, D. H. & Pause, A. (2014) Folliculin regulates ampk-dependent autophagy and metabolic stress survival, *PLoS genetics.* **10**, e1004273.
26. Gwinn, D. M., Shackelford, D. B., Egan, D. F., Mihaylova, M. M., Mery, A., Vasquez, D. S., Turk, B. E. & Shaw, R. J. (2008) AMPK phosphorylation of raptor mediates a metabolic checkpoint, *Molecular cell.* **30**, 214-26.
27. Shackelford, D. B. & Shaw, R. J. (2009) The LKB1-AMPK pathway: metabolism and growth control in tumour suppression, *Nature reviews Cancer.* **9**, 563-75.
28. Inoki, K., Ouyang, H., Zhu, T., Lindvall, C., Wang, Y., Zhang, X., Yang, Q., Bennett, C., Harada, Y., Stankunas, K., Wang, C. Y., He, X., MacDougald, O. A., You, M., Williams, B. O. & Guan, K. L. (2006) TSC2 integrates Wnt and energy signals via a coordinated phosphorylation by AMPK and GSK3 to regulate cell growth, *Cell.* **126**, 955-68.
29. Lizcano, J. M., Goransson, O., Toth, R., Deak, M., Morrice, N. A., Boudeau, J., Hawley, S. A., Udd, L., Makela, T. P., Hardie, D. G. & Alessi, D. R. (2004) LKB1 is a master kinase that activates 13 kinases of the AMPK subfamily, including MARK/PAR-1, *The EMBO journal.* **23**, 833-43.
30. Shaw, R. J., Bardeesy, N., Manning, B. D., Lopez, L., Kosmatka, M., DePinho, R. A. & Cantley, L. C. (2004) The LKB1 tumor suppressor negatively regulates mTOR signaling, *Cancer cell.* **6**, 91-9.
31. Shaw, R. J., Kosmatka, M., Bardeesy, N., Hurley, R. L., Witters, L. A., DePinho, R. A. & Cantley, L. C. (2004) The tumor suppressor LKB1 kinase directly activates AMP-activated kinase and regulates apoptosis in response to energy stress, *Proceedings of the National Academy of Sciences of the United States of America.* **101**, 3329-35.
32. Pineda, C. T., Ramanathan, S., Fon Tacer, K., Weon, J. L., Potts, M. B., Ou, Y. H., White, M. A. & Potts, P. R. (2015) Degradation of AMPK by a cancer-specific ubiquitin ligase, *Cell.* **160**, 715-28.
33. Woods, A., Dickerson, K., Heath, R., Hong, S. P., Momcilovic, M., Johnstone, S. R., Carlson, M. & Carling, D. (2005) Ca2+/calmodulin-dependent protein kinase kinase-beta acts upstream of AMP-activated protein kinase in mammalian cells, *Cell metabolism.* **2**, 21-33.
34. Memmott, R. M., Gills, J. J., Hollingshead, M., Powers, M. C., Chen, Z., Kemp, B., Kozikowski, A. & Dennis, P. A. (2008) Phosphatidylinositol ether lipid analogues induce AMP-activated protein kinase-dependent death in LKB1-mutant non small cell lung cancer cells, *Cancer research.* **68**, 580-8.
35. Goransson, O., McBride, A., Hawley, S. A., Ross, F. A., Shpiro, N., Foretz, M., Viollet, B., Hardie, D. G. & Sakamoto, K. (2007) Mechanism of action of A-769662, a valuable tool for activation of AMP-activated protein kinase, *The Journal of biological chemistry.* **282**, 32549-60.
36. Momcilovic, M., Hong, S. P. & Carlson, M. (2006) Mammalian TAK1 activates Snf1 protein kinase in yeast and phosphorylates AMP-activated protein kinase in vitro, *The Journal of biological chemistry.* **281**, 25336-43.
37. Mungai, P. T., Waypa, G. B., Jairaman, A., Prakriya, M., Dokic, D., Ball, M. K. & Schumacker, P. T. (2011) Hypoxia triggers AMPK activation through reactive oxygen species-mediated activation of calcium release-activated calcium channels, *Molecular and cellular biology.* **31**, 3531-45.

38. Popovics, P., Frigo, D. E., Schally, A. V. & Rick, F. G. (2015) Targeting the 5'-AMP-activated protein kinase and related metabolic pathways for the treatment of prostate cancer, *Expert opinion on therapeutic targets.* **19**, 617-32.
39. Hardie, D. G. (2015) Molecular Pathways: Is AMPK a Friend or a Foe in Cancer?, *Clinical cancer research : an official journal of the American Association for Cancer Research*.
40. Gao, J., Aksoy, B. A., Dogrusoz, U., Dresdner, G., Gross, B., Sumer, S. O., Sun, Y., Jacobsen, A., Sinha, R., Larsson, E., Cerami, E., Sander, C. & Schultz, N. (2013) Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal, *Science signaling.* **6**, pl1.
41. Cerami, E., Gao, J., Dogrusoz, U., Gross, B. E., Sumer, S. O., Aksoy, B. A., Jacobsen, A., Byrne, C. J., Heuer, M. L., Larsson, E., Antipin, Y., Reva, B., Goldberg, A. P., Sander, C. & Schultz, N. (2012) The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data, *Cancer discovery.* **2**, 401-4.
42. Barretina, J., Caponigro, G., Stransky, N., Venkatesan, K., Margolin, A. A., Kim, S., Wilson, C. J., Lehar, J., Kryukov, G. V., Sonkin, D., Reddy, A., Liu, M., Murray, L., Berger, M. F., Monahan, J. E., Morais, P., Meltzer, J., Korejwa, A., Jane-Valbuena, J., Mapa, F. A., Thibault, J., Bric-Furlong, E., Raman, P., Shipway, A., Engels, I. H., Cheng, J., Yu, G. K., Yu, J., Aspesi, P., Jr., de Silva, M., Jagtap, K., Jones, M. D., Wang, L., Hatton, C., Palescandolo, E., Gupta, S., Mahan, S., Sougnez, C., Onofrio, R. C., Liefeld, T., MacConaill, L., Winckler, W., Reich, M., Li, N., Mesirov, J. P., Gabriel, S. B., Getz, G., Ardlie, K., Chan, V., Myer, V. E., Weber, B. L., Porter, J., Warmuth, M., Finan, P., Harris, J. L., Meyerson, M., Golub, T. R., Morrissey, M. P., Sellers, W. R., Schlegel, R. & Garraway, L. A. (2012) The Cancer Cell Line Encyclopedia enables predictive modelling of anticancer drug sensitivity, *Nature.* **483**, 603-7.
43. Sakamoto, K., Zarrinpashneh, E., Budas, G. R., Pouleur, A. C., Dutta, A., Prescott, A. R., Vanoverschelde, J. L., Ashworth, A., Jovanovic, A., Alessi, D. R. & Bertrand, L. (2006) Deficiency of LKB1 in heart prevents ischemia-mediated activation of AMPKalpha2 but not AMPKalpha1, *American journal of physiology Endocrinology and metabolism.* **290**, E780-8.
44. Miyamoto, T., Rho, E., Sample, V., Akano, H., Magari, M., Ueno, T., Gorshkov, K., Chen, M., Tokumitsu, H., Zhang, J. & Inoue, T. (2015) Compartmentalized AMPK signaling illuminated by genetically encoded molecular sensors and actuators, *Cell reports.* **11**, 657-70.
45. Trojan, J., Brieger, A., Raedle, J., Esteller, M. & Zeuzem, S. (2000) 5'-CpG island methylation of the LKB1/STK11 promoter and allelic loss at chromosome 19p13.3 in sporadic colorectal cancer, *Gut.* **47**, 272-6.
46. Shackelford, D. B., Abt, E., Gerken, L., Vasquez, D. S., Seki, A., Leblanc, M., Wei, L., Fishbein, M. C., Czernin, J., Mischel, P. S. & Shaw, R. J. (2013) LKB1 inactivation dictates therapeutic response of non-small cell lung cancer to the metabolism drug phenformin, *Cancer cell.* **23**, 143-58.
47. Dupuy, F., Griss, T., Blagih, J., Bridon, G., Avizonis, D., Ling, C., Dong, Z., Siwak, D. R., Annis, M. G., Mills, G. B., Muller, W. J., Siegel, P. M. & Jones, R. G. (2013) LKB1 is a central regulator of tumor initiation and pro-growth metabolism in ErbB2-mediated breast cancer, *Cancer & metabolism.* **1**, 18.
48. Faubert, B., Boily, G., Izreig, S., Griss, T., Samborska, B., Dong, Z., Dupuy, F., Chambers, C., Fuerth, B. J., Viollet, B., Mamer, O. A., Avizonis, D., DeBerardinis, R. J., Siegel, P. M. & Jones, R. G. (2013) AMPK is a negative regulator of the Warburg effect and suppresses tumor growth in vivo, *Cell metabolism.* **17**, 113-24.
49. Momcilovic, M. & Shackelford, D. B. (2015) Targeting LKB1 in cancer - exposing and exploiting vulnerabilities, *British journal of cancer*.
50. Faubert, B., Vincent, E. E., Poffenberger, M. C. & Jones, R. G. (2015) The AMP-activated protein kinase (AMPK) and cancer: many faces of a metabolic regulator, *Cancer letters.* **356**, 165-70.
51. Bright, N. J., Thornton, C. & Carling, D. (2009) The regulation and function of mammalian AMPK-related kinases, *Acta physiologica.* **196**, 15-26.

52. McDonald, J. A. (2014) Canonical and noncanonical roles of Par-1/MARK kinases in cell migration, *International review of cell and molecular biology.* **312**, 169-99.
53. Suzuki, A., Ogura, T. & Esumi, H. (2006) NDR2 acts as the upstream kinase of ARK5 during insulin-like growth factor-1 signaling, *The Journal of biological chemistry.* **281**, 13915-21.
54. Hurov, J. B., Watkins, J. L. & Piwnica-Worms, H. (2004) Atypical PKC phosphorylates PAR-1 kinases to regulate localization and activity, *Current biology : CB.* **14**, 736-41.
55. Halford, N. G. & Hey, S. J. (2009) Snf1-related protein kinases (SnRKs) act within an intricate network that links metabolic and stress signalling in plants, *The Biochemical journal.* **419**, 247-59.
56. Kusakai, G., Suzuki, A., Ogura, T., Kaminishi, M. & Esumi, H. (2004) Strong association of ARK5 with tumor invasion and metastasis, *Journal of experimental & clinical cancer research : CR.* **23**, 263-8.
57. Suzuki, A., Lu, J., Kusakai, G., Kishimoto, A., Ogura, T. & Esumi, H. (2004) ARK5 is a tumor invasion-associated factor downstream of Akt signaling, *Molecular and cellular biology.* **24**, 3526-35.
58. Zagorska, A., Deak, M., Campbell, D. G., Banerjee, S., Hirano, M., Aizawa, S., Prescott, A. R. & Alessi, D. R. (2010) New roles for the LKB1-NUAK pathway in controlling myosin phosphatase complexes and cell adhesion, *Science signaling.* **3**, ra25.
59. Bell, R. E., Khaled, M., Netanely, D., Schubert, S., Golan, T., Buxbaum, A., Janas, M. M., Postolsky, B., Goldberg, M. S., Shamir, R. & Levy, C. (2014) Transcription factor/microRNA axis blocks melanoma invasion program by miR-211 targeting NUAK1, *The Journal of investigative dermatology.* **134**, 441-51.
60. Benaich, N., Woodhouse, S., Goldie, S. J., Mishra, A., Quist, S. R. & Watt, F. M. (2014) Rewiring of an epithelial differentiation factor, miR-203, to inhibit human squamous cell carcinoma metastasis, *Cell reports.* **9**, 104-17.
61. Riester, M., Wei, W., Waldron, L., Culhane, A. C., Trippa, L., Oliva, E., Kim, S. H., Michor, F., Huttenhower, C., Parmigiani, G. & Birrer, M. J. (2014) Risk prediction for late-stage ovarian cancer by meta-analysis of 1525 patient samples, *Journal of the National Cancer Institute.* **106**.
62. Dulak, A. M., Stojanov, P., Peng, S., Lawrence, M. S., Fox, C., Stewart, C., Bandla, S., Imamura, Y., Schumacher, S. E., Shefler, E., McKenna, A., Carter, S. L., Cibulskis, K., Sivachenko, A., Saksena, G., Voet, D., Ramos, A. H., Auclair, D., Thompson, K., Sougnez, C., Onofrio, R. C., Guiducci, C., Beroukhim, R., Zhou, Z., Lin, L., Lin, J., Reddy, R., Chang, A., Landrenau, R., Pennathur, A., Ogino, S., Luketich, J. D., Golub, T. R., Gabriel, S. B., Lander, E. S., Beer, D. G., Godfrey, T. E., Getz, G. & Bass, A. J. (2013) Exome and whole-genome sequencing of esophageal adenocarcinoma identifies recurrent driver events and mutational complexity, *Nature genetics.* **45**, 478-86.
63. Liu, L., Ulbrich, J., Muller, J., Wustefeld, T., Aeberhard, L., Kress, T. R., Muthalagu, N., Rycak, L., Rudalska, R., Moll, R., Kempa, S., Zender, L., Eilers, M. & Murphy, D. J. (2012) Deregulated MYC expression induces dependence upon AMPK-related kinase 5, *Nature.* **483**, 608-12.
64. Goga, A., Samson, S. & D. Horiuchi (2012). Identification of Novel Synthetic-Lethal Targets for MYC-Driven Triple-Negative Breast Cancer. Paper presented at the *Thirty-Fifth Annual CTRC – AACR San Antonio Breast Cancer Symposium*, San Antonia, TX.
65. Banerjee, S., Buhrlage, S. J., Huang, H. T., Deng, X., Zhou, W., Wang, J., Traynor, R., Prescott, A. R., Alessi, D. R. & Gray, N. S. (2014) Characterization of WZ4003 and HTH-01-015 as selective inhibitors of the LKB1-tumour-suppressor-activated NUAK kinases, *The Biochemical journal.* **457**, 215-25.
66. Li, F., Wang, Y., Zeller, K. I., Potter, J. J., Wonsey, D. R., O'Donnell, K. A., Kim, J. W., Yustein, J. T., Lee, L. A. & Dang, C. V. (2005) Myc stimulates nuclearly encoded mitochondrial genes and mitochondrial biogenesis, *Molecular and cellular biology.* **25**, 6225-34.
67. Kessler, J. D., Kahle, K. T., Sun, T., Meerbrey, K. L., Schlabach, M. R., Schmitt, E. M., Skinner, S. O., Xu, Q., Li, M. Z., Hartman, Z. C., Rao, M., Yu, P., Dominguez-Vidana, R., Liang, A. C., Solimini,

N. L., Bernardi, R. J., Yu, B., Hsu, T., Golding, I., Luo, J., Osborne, C. K., Creighton, C. J., Hilsenbeck, S. G., Schiff, R., Shaw, C. A., Elledge, S. J. & Westbrook, T. F. (2012) A SUMOylation-dependent transcriptional subprogram is required for Myc-driven tumorigenesis, *Science.* **335**, 348-53.
68. Fischer, U. & Meese, E. (2007) Glioblastoma multiforme: the role of DSB repair between genotype and phenotype, *Oncogene.* **26**, 7809-15.
69. Namiki, T., Tanemura, A., Valencia, J. C., Coelho, S. G., Passeron, T., Kawaguchi, M., Vieira, W. D., Ishikawa, M., Nishijima, W., Izumo, T., Kaneko, Y., Katayama, I., Yamaguchi, Y., Yin, L., Polley, E. C., Liu, H., Kawakami, Y., Eishi, Y., Takahashi, E., Yokozeki, H. & Hearing, V. J. (2011) AMP kinase-related kinase NUAK2 affects tumor growth, migration, and clinical outcome of human melanoma, *Proceedings of the National Academy of Sciences of the United States of America.* **108**, 6597-602.
70. Mesquita, B., Lopes, P., Rodrigues, A., Pereira, D., Afonso, M., Leal, C., Henrique, R., Lind, G. E., Jeronimo, C., Lothe, R. A. & Teixeira, M. R. (2013) Frequent copy number gains at 1q21 and 1q32 are associated with overexpression of the ETS transcription factors ETV3 and ELF3 in breast cancer irrespective of molecular subtypes, *Breast cancer research and treatment.* **138**, 37-45.
71. Riemenschneider, M. J., Buschges, R., Wolter, M., Reifenberger, J., Bostrom, J., Kraus, J. A., Schlegel, U. & Reifenberger, G. (1999) Amplification and overexpression of the MDM4 (MDMX) gene from 1q32 in a subset of malignant gliomas without TP53 mutation or MDM2 amplification, *Cancer research.* **59**, 6091-6.
72. Lefebvre, D. L. & Rosen, C. F. (2005) Regulation of SNARK activity in response to cellular stresses, *Biochimica et biophysica acta.* **1724**, 71-85.
73. Tsuchihara, K., Ogura, T., Fujioka, R., Fujii, S., Kuga, W., Saito, M., Ochiya, T., Ochiai, A. & Esumi, H. (2008) Susceptibility of Snark-deficient mice to azoxymethane-induced colorectal tumorigenesis and the formation of aberrant crypt foci, *Cancer science.* **99**, 677-82.
74. Hurov, J. & Piwnica-Worms, H. (2007) The Par-1/MARK family of protein kinases: from polarity to metabolism, *Cell cycle.* **6**, 1966-9.
75. Sun, C., Tian, L., Nie, J., Zhang, H., Han, X. & Shi, Y. (2012) Inactivation of MARK4, an AMP-activated protein kinase (AMPK)-related kinase, leads to insulin hypersensitivity and resistance to diet-induced obesity, *The Journal of biological chemistry.* **287**, 38305-15.
76. Hurov, J. B., Huang, M., White, L. S., Lennerz, J., Choi, C. S., Cho, Y. R., Kim, H. J., Prior, J. L., Piwnica-Worms, D., Cantley, L. C., Kim, J. K., Shulman, G. I. & Piwnica-Worms, H. (2007) Loss of the Par-1b/MARK2 polarity kinase leads to increased metabolic rate, decreased adiposity, and insulin hypersensitivity in vivo, *Proceedings of the National Academy of Sciences of the United States of America.* **104**, 5680-5.
77. Lennerz, J. K., Hurov, J. B., White, L. S., Lewandowski, K. T., Prior, J. L., Planer, G. J., Gereau, R. W. t., Piwnica-Worms, D., Schmidt, R. E. & Piwnica-Worms, H. (2010) Loss of Par-1a/MARK3/C-TAK1 kinase leads to reduced adiposity, resistance to hepatic steatosis, and defective gluconeogenesis, *Molecular and cellular biology.* **30**, 5043-56.
78. Gallagher, E. J. & LeRoith, D. (2015) Obesity and Diabetes: The Increased Risk of Cancer and Cancer-Related Mortality, *Physiological reviews.* **95**, 727-748.
79. Goodwin, J. M., Svensson, R. U., Lou, H. J., Winslow, M. M., Turk, B. E. & Shaw, R. J. (2014) An AMPK-independent signaling pathway downstream of the LKB1 tumor suppressor controls Snail1 and metastatic potential, *Molecular cell.* **55**, 436-50.
80. Hubaux, R., Thu, K. L., Vucic, E. A., Pikor, L. A., Kung, S. H., Martinez, V. D., Mosslemi, M., Becker-Santos, D. D., Gazdar, A. F., Lam, S. & Lam, W. L. (2015) Microtubule affinity-regulating kinase 2 is associated with DNA damage response and cisplatin resistance in non-small cell lung cancer, *International journal of cancer Journal international du cancer*.
81. Cancer Genome Atlas Research, N. (2014) Comprehensive molecular profiling of lung adenocarcinoma, *Nature.* **511**, 543-50.

82. Wu, Z. Z., Lu, H. P. & Chao, C. C. (2010) Identification and functional analysis of genes which confer resistance to cisplatin in tumor cells, *Biochemical pharmacology.* **80**, 262-76.
83. Ossipova, O., Dhawan, S., Sokol, S. & Green, J. B. (2005) Distinct PAR-1 proteins function in different branches of Wnt signaling during vertebrate development, *Developmental cell.* **8**, 829-41.
84. Myant, K. & Sansom, O. J. (2011) Wnt/Myc interactions in intestinal cancer: partners in crime, *Experimental cell research.* **317**, 2725-31.
85. Anastas, J. N. & Moon, R. T. (2013) WNT signalling pathways as therapeutic targets in cancer, *Nature reviews Cancer.* **13**, 11-26.
86. Kodaka, M. & Hata, Y. (2015) The mammalian Hippo pathway: regulation and function of YAP1 and TAZ, *Cellular and molecular life sciences : CMLS.* **72**, 285-306.
87. Moroishi, T., Hansen, C. G. & Guan, K. L. (2015) The emerging roles of YAP and TAZ in cancer, *Nature reviews Cancer.* **15**, 73-9.
88. Humbert, N., Navaratnam, N., Augert, A., Da Costa, M., Martien, S., Wang, J., Martinez, D., Abbadie, C., Carling, D., de Launoit, Y., Gil, J. & Bernard, D. (2010) Regulation of ploidy and senescence by the AMPK-related kinase NUAK1, *The EMBO journal.* **29**, 376-86.
89. Mohseni, M., Sun, J., Lau, A., Curtis, S., Goldsmith, J., Fox, V. L., Wei, C., Frazier, M., Samson, O., Wong, K. K., Kim, C. & Camargo, F. D. (2014) A genetic screen identifies an LKB1-MARK signalling axis controlling the Hippo-YAP pathway, *Nature cell biology.* **16**, 108-17.
90. Mo, J. S., Meng, Z., Kim, Y. C., Park, H. W., Hansen, C. G., Kim, S., Lim, D. S. & Guan, K. L. (2015) Cellular energy stress induces AMPK-mediated regulation of YAP and the Hippo pathway, *Nature cell biology.* **17**, 500-10.
91. Wang, W., Xiao, Z. D., Li, X., Aziz, K. E., Gan, B., Johnson, R. L. & Chen, J. (2015) AMPK modulates Hippo pathway activity to regulate energy homeostasis, *Nature cell biology.* **17**, 490-9.
92. DeRan, M., Yang, J., Shen, C. H., Peters, E. C., Fitamant, J., Chan, P., Hsieh, M., Zhu, S., Asara, J. M., Zheng, B., Bardeesy, N., Liu, J. & Wu, X. (2014) Energy stress regulates hippo-YAP signaling involving AMPK-mediated regulation of angiomotin-like 1 protein, *Cell reports.* **9**, 495-503.
93. Hardie, D. G. (2014) AMPK--sensing energy while talking to other signaling pathways, *Cell metabolism.* **20**, 939-52.

Figure 1

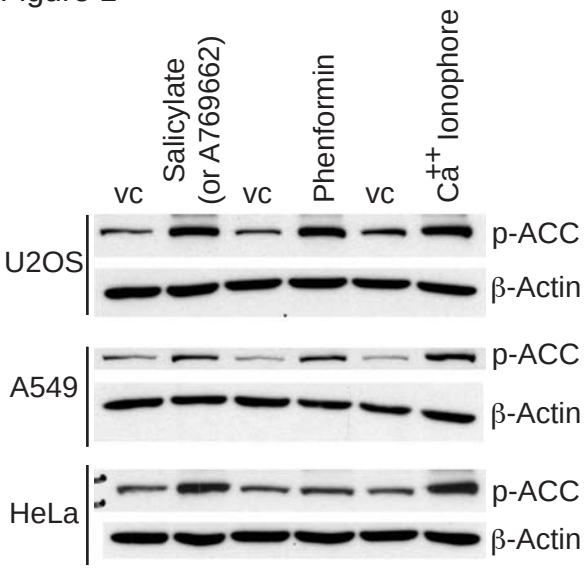

vc
Salicylate (or A769662)
vc
Phenformin
vc
Ca++ Ionophore
U2OS
p-ACC
β-Actin
A549
p-ACC
β-Actin
HeLa
p-ACC
β-Actin

Figure 2

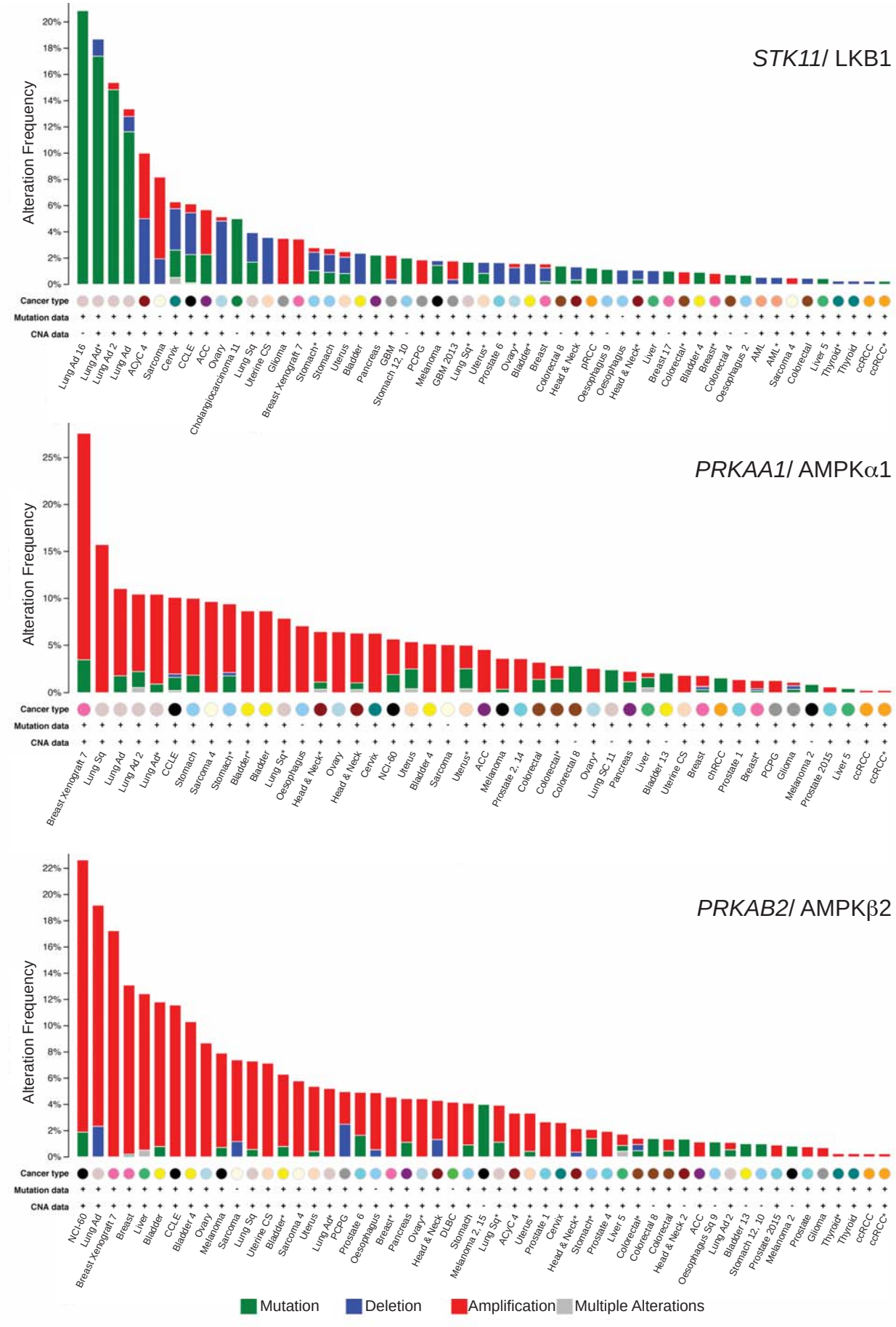
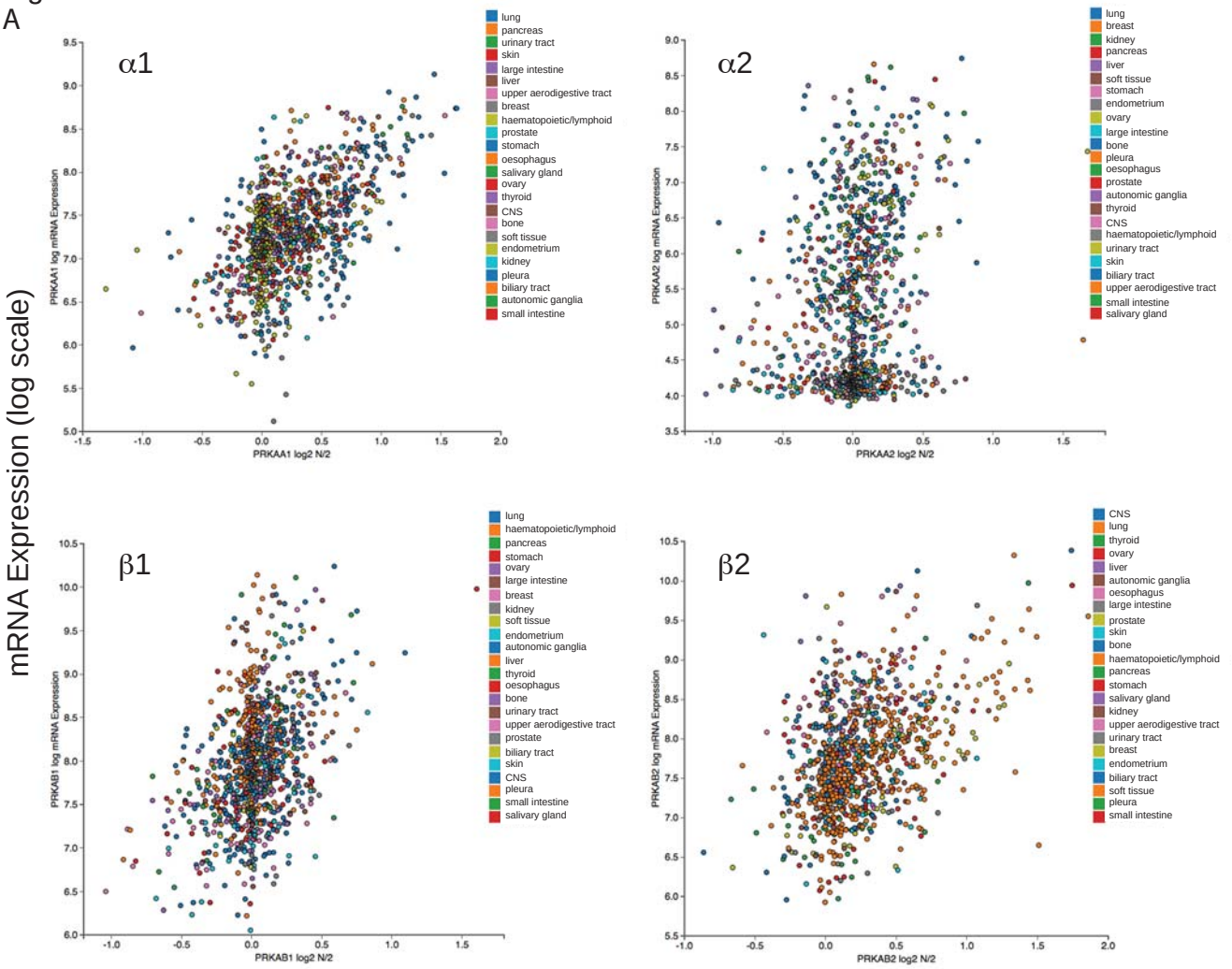
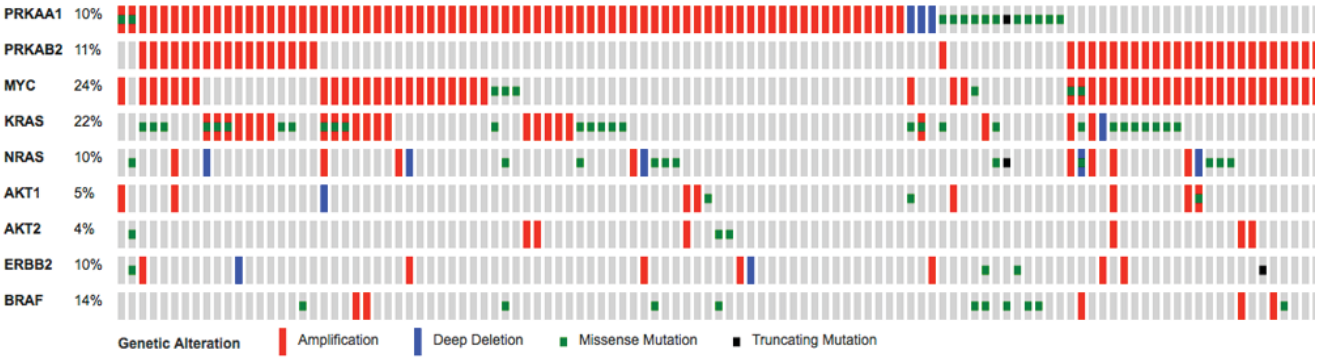

STK11/ LKB1
PRKAA1/ AMPKα1
PRKAB2/ AMPKβ2
Alteration Frequency
Cancer type
Mutation data
CNA data
Mutation
Deletion
Amplification
Multiple Alterations

Figure 3

A



B



C

Gene A	Gene B	p-Value	Log Odds Ratio	Association	
PRKAA1	KRAS	<0.001	0.886	Tendency towards co-occurrence	Significant
PRKAB2	MYC	<0.001	0.960	Tendency towards co-occurrence	Significant
PRKAB2	KRAS	<0.001	0.800	Tendency towards co-occurrence	Significant
MYC	KRAS	<0.001	0.625	Tendency towards co-occurrence	Significant
NRAS	AKT1	<0.001	1.297	Tendency towards co-occurrence	Significant
PRKAB2	NRAS	0.004	0.814	Tendency towards co-occurrence	Significant
PRKAA1	PRKAB2	0.009	0.759	Tendency towards co-occurrence	Significant

Figure 4

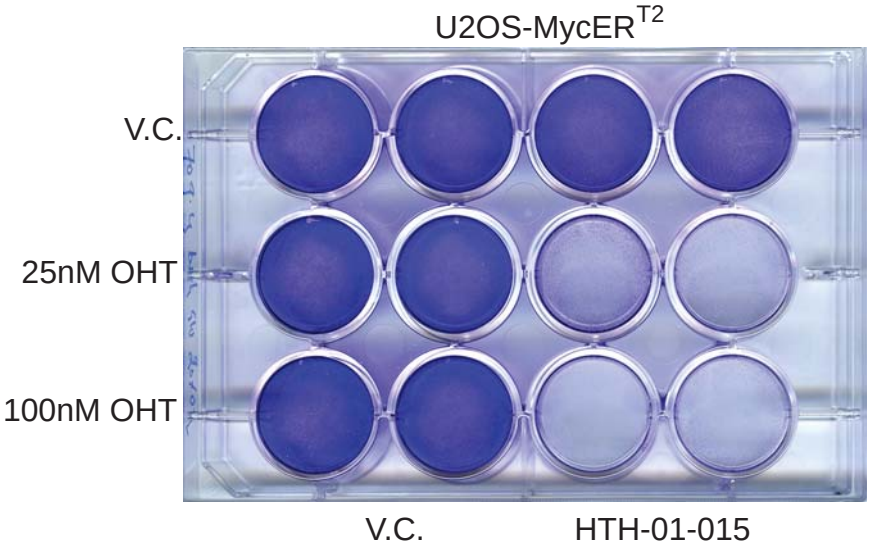


Figure 5

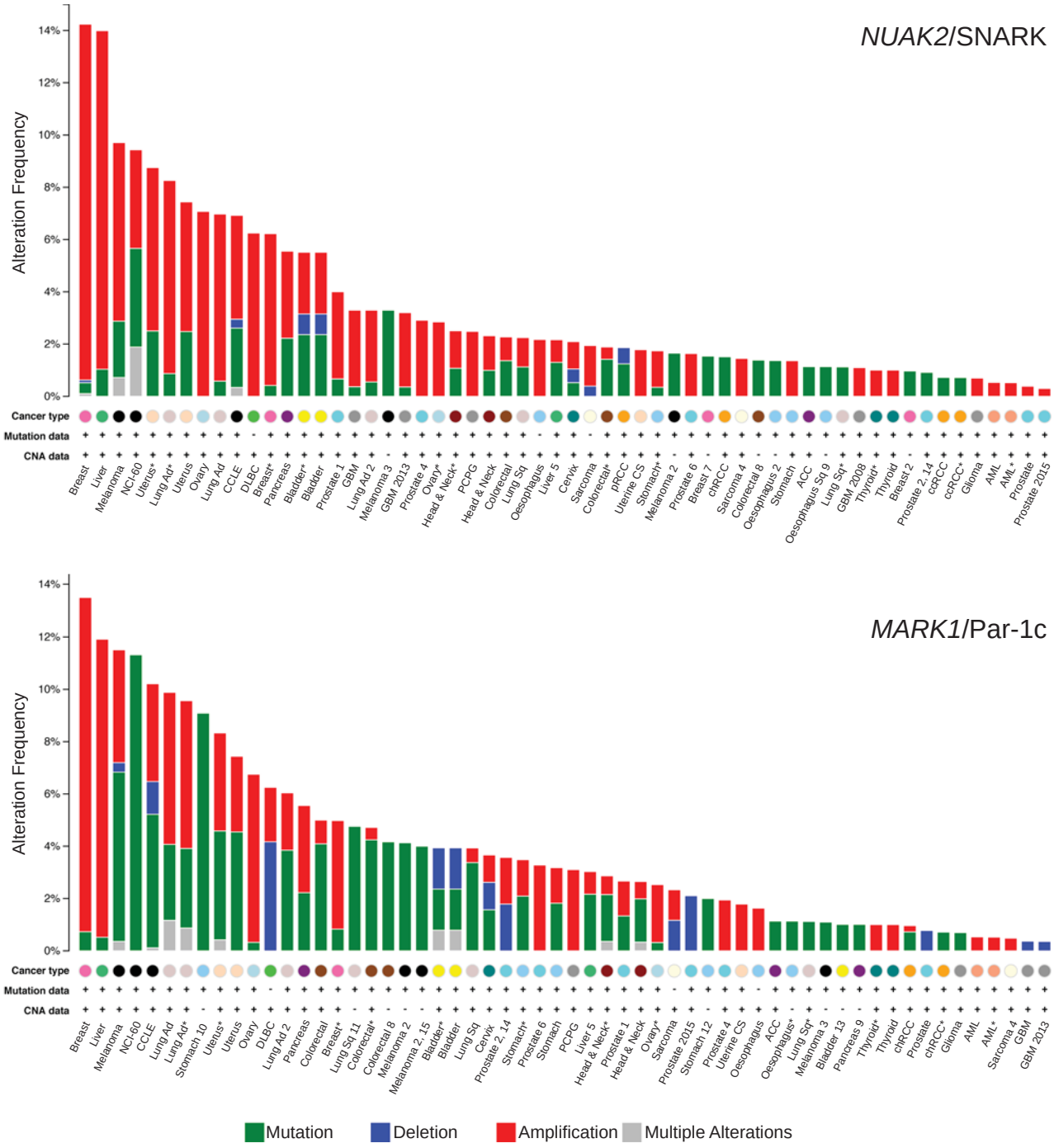
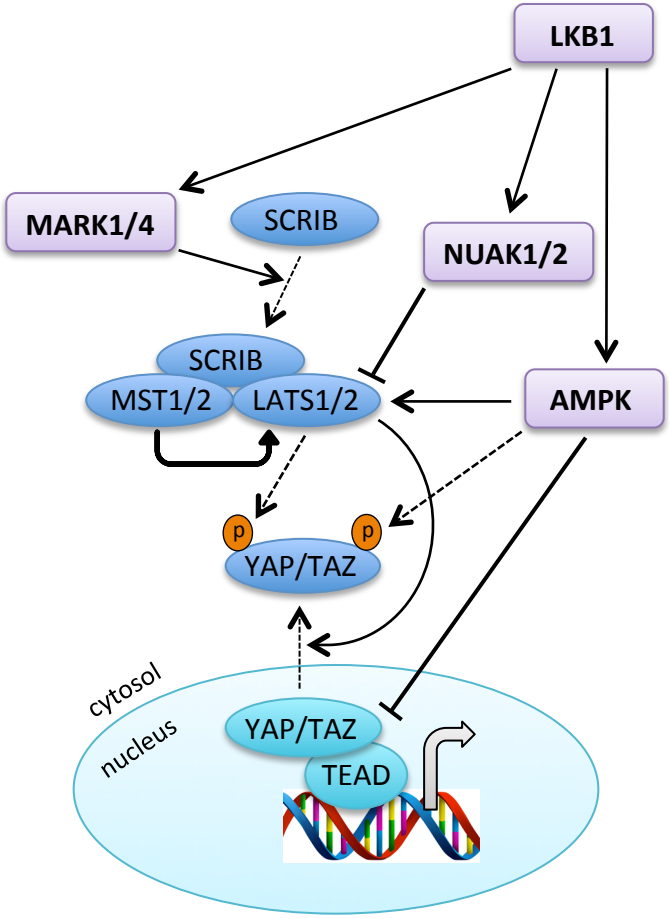

NUAK2/SNARK
Alteration Frequency
14%
12%
10%
8%
6%
4%
2%
0%
Cancer type
Mutation data
CNA data
Breast
Liver
Melanoma
NCI-60
Uterus*
Lung Ad*
Uterus
Ovary
Lung Ad
CCLE
DLBC
Breast*
Pancreas
Bladder*
Bladder
Prostate 1
GBM
Lung Ad 2
Melanoma 3
GBM 2013
Prostate 4
Ovary*
Head & Neck*
PCPG
Head & Neck
Colorectal
Lung Sq
Oesophagus
Liver 5
Cervix
Sarcoma
Colorectal*
pRCC
Uterine CS
Stomach*
Melanoma 2
Prostate 6
Breast 7
chRCC
Sarcoma 4
Colorectal 8
Oesophagus 2
Stomach
ACC
Oesophagus Sq 9
Lung Sq*
GBM 2008
Thyroid*
Thyroid
Breast 2
Prostate 2, 14
ccRCC
ccRCC*
Glioma
AML
AML*
Prostate
Prostate 2015
MARK1/Par-1c
Alteration Frequency
14%
12%
10%
8%
6%
4%
2%
0%
Cancer type
Mutation data
CNA data
Breast
Liver
Melanoma
NCI-60
CCLE
Lung Ad
Lung Ad*
Stomach 10
Uterus*
Uterus
Ovary
DLBC
Lung Ad 2
Pancreas
Colorectal
Breast*
Lung Sq 11
Colorectal*
Colorectal 8
Melanoma 2
Melanoma 2, 15
Bladder*
Bladder
Lung Sq
Cervix
Prostate 2, 14
Stomach*
Prostate 6
Stomach
PCPG
Liver 5
Head & Neck*
Prostate 1
Head & Neck
Ovary*
Sarcoma
Prostate 2015
Stomach 12
Prostate 4
Uterine CS
Oesophagus
ACC
Oesophagus*
Lung Sq*
Melanoma 3
Bladder 13
Pancreas 9
Thyroid*
Thyroid
chRCC
Prostate
chRCC*
Glioma
AML
AML*
Sarcoma 4
GBM
GBM 2013
Mutation
Deletion
Amplification
Multiple Alterations

Figure 6


LKB1
MARK1/4
SCRIB
NUAK1/2
SCRIB
MST1/2
LATS1/2
AMPK
p
p
YAP/TAZ
cytosol
nucleus
YAP/TAZ
TEAD